

Supplementary Table 2. All strains and corresponding genotypes relevant in this study. The generalized denotation and genotype of strains used in GFP characterization, containing *esal* expression cassettes with different promoter/RBS combinations, is given as "LXX". Strains used for metabolite production experiments are denoted with their specific *esal* expression cassettes and genotype, with the predicted *esal* expression strength presented as a percentage of the maximum in the library provided in Mutualik et al¹⁰.

Strain	Genotype	Description
QS-based transcriptional control of GFP expression (P_{esas})		
AG2681	MG1655 <i>HK022::apFAB104-EsaRI70V</i>	EsaRI70V under control of constitutive promoter
LXX	MG1655 <i>HK022::apFAB104-EsaRI70V 186(O)::apFABXX-apFABXX-esal</i>	AG2681 + Esal driven by a specific promoter and RBS, generalized as "apFABXX" here. The full list of promoter-RBS combinations is given in Supplementary Table 1. For GFP characterization (Fig. 1B and 1C), this strain series was transformed with pCOLA- P_{esas} -GFP(LVA)
QS-based transcriptional control for metabolite production (P_{esas})		
IB1379	MG1655 $\Delta endA \Delta zwf \Delta pfkB$	Wildtype pfkA promoter control
IB2275	MG1655 $\Delta endA \Delta zwf \Delta pfkB pfkA::P_{esas}-pfkA(LAA) HK022::104-esaRI170V$	Transcriptional control of tagged pfkA under P_{esas} promoter; esaRI70V under control of a synthetic promoter (apFAB104)
L24S	MG1655 $\Delta endA \Delta zwf \Delta pfkB pfkA::P_{esas}-pfkA(LAA) HK022::104-esaRI170V 186(O)::apFAB295-apFAB699-esal$	IB2275 + esal driven by selected promoter and RBS sequences from Mutualik et al; Predicted strength: 4.8%

L30S	MG1655 $\Delta endA \Delta zwf \Delta pfkB pfkA::P_{esaS}$ - $pfkA(LAA)$ HK022::104- $esaRI170V 186(O)::apFAB65-apFAB699-esal$	See description for L24S; Predicted strength: 2.9%
L19S	MG1655 $\Delta endA \Delta zwf \Delta pfkB pfkA::P_{esaS}$ - $pfkA(LAA)$ HK022::104- $esaRI170V 186(O)::apFAB296-apFAB700-esal$	See description for L24S; Predicted strength: 1.7%
L25S	MG1655 $\Delta endA \Delta zwf \Delta pfkB pfkA::P_{esaS}$ - $pfkA(LAA)$ HK022::104- $esaRI170V 186(O)::apFAB295-apFAB700-esal$	See description for L24S; Predicted strength: 1.3%
L31S	MG1655 $\Delta endA \Delta zwf \Delta pfkB pfkA::P_{esaS}$ - $pfkA(LAA)$ HK022::104- $esaRI170V 186(O)::apFAB65-apFAB700-esal$	See description for L24S; Predicted strength: 1.2%
B1379GA	MG1655(DE3) $\Delta endA \Delta zwf \Delta pfkB \Delta uxaC \Delta gudD$	IB1379 + λ DE3 lysogen + $uxaC$ and $gudD$ knockout
L24GA	MG1655(DE3) $\Delta endA \Delta zwf \Delta pfkB \Delta uxaC \Delta gudD pfkA::P_{esaS}$ - $pfkA(LAA)$ HK022::104- $esaRI170V 186(O)::apFAB295-apFAB699$ - $esal$	L24S + λ DE3 lysogen + $uxaC$ and $gudD$ knockout
L19GA	MG1655(DE3) $\Delta endA \Delta zwf \Delta pfkB \Delta uxaC \Delta gudD pfkA::P_{esaS}$ - $pfkA(LAA)$ HK022::104- $esaRI170V 186(O)::apFAB296-apFAB700$ - $esal$	See description for L24SGA
L31GA	MG1655(DE3) $\Delta endA \Delta zwf \Delta pfkB \Delta uxaC \Delta gudD pfkA::P_{esaS}$ - $pfkA(LAA)$ HK022::104- $esaRI170V 186(O)::apFAB65-apFAB700$ - $esal$	See description for L24SGA
AG2310	MG1655 $\Delta aroK \Delta aroL$ HK022:: $esaRI170V-P_{esaS}$ - $aroK(ASV)$	Native shikimate kinases deleted and $aroK$ under the control of the P_{esaS} promoter
L24SA	MG1655 $\Delta aroK \Delta aroL$ HK022:: $esaRI170V-P_{esaS}$ - $aroK(ASV)$ 180(O):: $apFAB295-apFAB699-esal$	AG + esal driven by selected promoter and RBS sequences from Mutualik et al; Predicted strength: 4.8%
L19SA	MG1655 $\Delta aroK \Delta aroL$ HK022:: $esaRI170V-P_{esaS}$ - $aroK(ASV)$ 186(O):: $apFAB296-apFAB700-esal$	See description for L24SA; Predicted strength: 1.7%

L25SA	MG1655 Δ a _{ro} K Δ a _{ro} L HK022::esaR _{I70V} -P _{esaS} -a _{ro} K(ASV) 186(O)::apFAB295-apFAB700-esaI	See description for L24SA; Predicted strength: 1.3%
L31SA	MG1655 Δ a _{ro} K Δ a _{ro} L HK022::esaR _{I70V} -P _{esaS} -a _{ro} K(ASV) 186(O)::apFAB65-apFAB700-esaI	See description for L24SA; Predicted strength: 1.2%
Phosphate-limited transcriptional control (P_{phoA})		
IB1863	MG1655 Δ endA Δ zwf Δ pfkB Δ sspB pfkA::114-pfkA(DAS+4) HK022::tetR-P _{tet} -sspB	pfkA with SsrA tag under control of a synthetic promoter (apFAB114); aTc-inducible promoter driving sspB expression
IB643	MG1655 Δ endA Δ zwf Δ pfkB Δ sspB pfkA::114-pfkA(DAS+4) HK022:: P _{phoA} -sspB	pfkA with SsrA tag under control of a synthetic promoter (apFAB114); Phosphate-starvation-inducible promoter driving sspB expression
IB1509	MG1655 Δ endA Δ zwf Δ pfkB Δ sspB pfkA::104-pfkA(DAS+4) HK022::P _{phoA} -sspB	pfkA with SsrA tag under control of a synthetic promoter (apFAB104); Phosphate-starvation-inducible promoter driving sspB expression
IB1624	MG1655 Δ endA Δ zwf Δ pfkB Δ sspB pfkA::104-pfkA(DAS+4)	pfkA with SsrA tag under control of a synthetic promoter (apFAB104)
QS-based post-translational control (P_{esaR})		
IB1643	MG1655 Δ endA Δ zwf Δ pfkB Δ sspB pfkA::114-pfkA(DAS+4)	pfkA with SsrA tag under control of a synthetic promoter (apFAB114)
IB2265	MG1655 Δ endA Δ zwf Δ pfkB Δ sspB pfkA::114-pfkA(DAS+4) HK022::esaR-P _{esaR} -sspB	IB1643 + sspB under control of the P _{esaR} promoter
AG2349	MG1655 Δ endA Δ zwf Δ pfkB Δ sspB pfkA::114-pfkA(DAS+4)HK022::esaR-P _{esaR} -tuned RBS-sspB	IB2265 background with optimized RBS to control sspB translation

L25R	MG1655 $\Delta endA \Delta zwf \Delta pfkB \Delta sspB pfkA::114-pfkA(DAS+4)$ $HK022::esaR-P_{esaR}$ -tuned RBS-sspB 186(O)::apFAB295-apFAB700-esal	AG2349 + esal driven by selected promoter and RBS sequences from Mutualik et al; Predicted strength: 1.3%
L31R	MG1655 $\Delta endA \Delta zwf \Delta pfkB \Delta sspB pfkA::114-pfkA(DAS+4)$ $HK022::EsaR-P_{esaR}$ -tuned RBS-sspB 186(O)::apFAB65-apFAB700-esal	See description for L25R; Predicted strength: 1.2%